IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Isacson, et al.
Title:	DOPAMINERGIC NEURONS DIFFERENTIATED FROM EMBRYONIC CELLS FOR TREATING NEURODEGENERATIVE DISEASES
Appl. No.:	10/731,550
Filing Date:	December 9, 2003
Examiner:	Zara, J.J.
Art Unit:	1635
Confirmation Number:	4567

PRE-APPEAL BRIEF REQUEST FOR REVIEW

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In accordance with the **Pre-Appeal Brief Conference Pilot Program**, this Pre-Appeal Brief Request is being filed together with a Notice of Appeal.

REMARKS

Claims 1, 3-6, 8, and 15-16 stand rejected for lack of enablement. Applicants respectfully traverse this rejection.

Claim 1, the sole pending independent claim, encompasses a method for the *in vitro* generation of dopaminergic neurons from pluripotent stem cells (e.g., embryonic stem cells) by inhibiting Smad4 (a TGF-β signaling pathway component) and overexpressing one or more cell fate-inducing polypeptides including, for example, PTX-3.

In several instances in the Final Office Action, the Examiner argues that the Specification does not enable generating dopaminergic neurons either by inhibiting any TGF-β pathway signaling component (see, for example, p. 3, ¶ 2) or generating such neurons "*in vitro* or *in vivo*" (see, for example, p. 3, ll. 2-4 and p. 4, ¶ 2). To the extent that these statements are indicative of an overbreadth rejection, Applicants point out that the claims have been limited by amendment to Smad4 inhibition and to generating dopaminergic neurons *in vitro*.

Smad4 Inhibition

The Specification fully enables generating dopaminergic neurons from pluripotent cells in which Smad4 is inhibited. The Examiner's attention is respectfully drawn to Example 1 in the Specification (pages 39-45). Here, Applicants describe a method for producing dopaminergic neurons from Smad4$^{-/-}$ embryonic stem cells. As shown in Figures 3-5 and the accompanying text at pages 43-45, the expression pattern of a variety of developmental markers was altered in the Smad$^{-/-}$ ES cells in a manner consistent with differentiation into dopaminergic neurons. The cells progressively lost mesodermal markers and gained certain general neuronal markers and dopaminergic markers. Thus, the Specification provides a working example which demonstrates that dopaminergic neurons can be generated *in vitro* from Smad4$^{-/-}$ ES cells.

The Specification teaches several other methods to enable Smad4 inhibition in pluripotent cells. The Specification describes the use of a Smad4 dominant negative protein (p. 30-31) and the use of Smad4 antisense nucleic acids (p. 24-26). With nothing more than routine skill, an artisan may practice either of these methods for Smad4 inhibition and apply them to the instant invention.

Furthermore, prior art of record recognizes several methods for effectively inhibiting Smad4 activity *in vitro*. A C-terminal truncation of Smad4 (e.g., Smad4(1-514)) results in a dominant negative protein, as taught by Simeone et al.,[1] Chen et al.,[2] and Zhang et al.[3] Effective

[1] Am. J. Physiol. Cell Physiol. 281: C311-C319, 2001. See, page C313 "Construction of Adenoviral Vectors" and Figure 7.

[2] J. Biol. Chem. 277: 36118-36128, 2002. See paragraph bridging pages 36119-36120.

[3] Am. J. Physiol. Gastrointest. Liver Physiol., 280: G1247-G1253, 2001. See page G1248, "Construction of Adenoviral Vectors".

Smad4 antisense nucleic acids are taught by Kretschmer et al.[4] and Zhao et al.[5] Thus, the prior art provides direction for multiple methods of Smad4 inhibition which document that methods in the Specification can be used for the *in vitro* inhibition of Smad4.

The Examiner questions the enablement of Smad4 inhibition but cites to no evidence or reasoning as to why she doubts Applicants' disclosure and the supporting evidence of the prior art discussed above. Applicants' Specification is presumed to be enabled and it is the Examiner's burden to come forward with acceptable evidence or reasoning as to why it is not. See MPEP 2164.04; See also Ex parte Hicks, 2000 WL 33673734 (Bd. Pat. App. & Interf., 2000). The rejection, however, improperly rests on Examiner's personal opinion. See, e.g., Final Office Action at p. 4, ¶ 2.

It is clear that the Examiner has failed to meet her burden to demonstrate a *prima facie* rejection for lack of enablement, and that the record is clear that a skilled artisan could readily inhibit Smad4 *in vitro* using human pluripotent cells at the time of application filing. Applicants submit that this basis for rejection is traversed.

Overexpression of a cell fate-inducing polypeptide

Turning next to the requirement for overexpression of one or more cell fate-inducing polypeptides, the Examiner alleges that undue experimentation would be required to transfect cell fate-inducing genes into human pluripotent cells (e.g., human ES cells). The Examiner argues that transfection of human pluripotent cells is unpredictable and bases this on the teachings of Zwaka et al.[6] and Odorico et al.[7] Final Office Action at p. 3; Office Action mailed January 31, 2007 at p. 7, ¶ 2. Specifically, the Examiner argues that Zwaka et al. show that high stable transfection efficiencies in human ES cells are difficult to achieve and that Odorico et al. document differences for *in vitro* culture requirements for maintaining ES cells from various animal species.

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[4] Oncogene, 22: 6748-6763, 2003. See page 6761, "GB Antisense Oligonucleotides" and Figure 6C.

[5] Dev. Biol., 194: 182-195. See paragraph bridging pages 183-184 and Figures 2 and 4.

[6] Nat. Biotechnol., 21: 319-321, 2003.

[7] Stem Cells, 19: 193-204, 2001.

This ground of rejection is based on a misunderstanding of the cited prior art. In fact, when the cited art is properly considered, Zwaka et al. and Odorico et al. support rather than refute the enablement of Applicants' claimed invention.

Zwaka et al. disclose that established mouse ES cell protocols inefficiently transfect human ES cells; yielding a stable transfection rate of about 10^{-7}. However, Zwaka et al. also demonstrate that, by performing nothing more than routine experimentation, the murine electroporation protocols may be optimized for human ES cells to achieve higher yields. Specifically, Zwaka et al. state that:

> [a]s human ES cells are significantly larger than mouse ES cells... we tried electroporation parameters described for larger cells. Additionally, we electroporated the cells in an isotonic, protein-rich solution (standard cell culture medium), instead of PBS, at room temperature. Using this modified protocol, we were able to obtain stable... transfection rates that were 100-fold (or more) higher than those attained with standard mouse ES cell electroporation procedures. Zwaka et al. at page 1, right column, first paragraph (emphasis added).
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> [e]lectroporation of human ES cells with a DNA construct containing a neo cassette under the control of the *tk* promoter yielded a stable transfection rate of 5.6×10^{-5}, giving an estimated 26:1 ratio of stable transfected clones to homologous recombination events for the first POU5F1 construct. Similarly, for transfection of the HPRT1 vector, the ratio of G418-resistant clones to $HPRT1^-$ clones was 50:1. Zwaka et al. at page 2, first paragraph (internal citations omitted) (emphasis added).

Zwaka et al., using standard techniques known in the art at the time of application filing, easily modified the murine-optimized electroporation protocols to significantly increase transfection efficiency in human ES cells.

As acknowledged by the Examiner, Odorico et al. provide a discussion of variables related to the successful culture of human ES cells. However, rather than proving that *in vitro* culture of human pluripotent cells is unpredictable, Odorico et al. provide the artisan with useful guidance that is applicable to Applicants' claimed invention. For example, Odorico et al. state:

> The [inner cell mass] cell outgrowths are propagated in the presence of serum and colonies with the appropriate undifferentiated morphology are subsequently selected and expended. After the initial derivation in serum, human ES cell lines can be maintained and propagated on feeder layers in medium containing serum

alone or serum replacement medium and basic fibroblast growth factor (bFGF). Odorico et al., at page 194, left column.

Thus, when properly considered, both prior art references relied on for the rejection actually support Applicants' case for enablement. Zwaka et al. provides important guidance on methods for modifying established murine transfection protocols in order to obtain comparable transfection rates in human cells. Odorico et al. provides significant guidance on the use of feeder cell layers and culture medium for maintaining pluripotent cells. Each of these references was available to the artisan at the time of application filing. Accordingly, the information provided therein was readily available to the artisan and may be usefully applied to the practice of Applicants' claimed invention.

In sum, the Specification, and the knowledge of the artisan at the time of application filing, fully enables one to make and use Applicants' claimed invention. The Specification, examples and teachings, and the prior art provide ample guidance for inhibiting Smad4, which evidence has not been contradicted by any acceptable evidence or reasoning of record. And, the Examiner's allegations of unpredictability based on Zwaka et al. and Odorico et al. are based on an unduly narrow and strained interpretation that ignores the references' teachings as a whole. In fact, the prior art relied upon by the Examiner, and other prior art of record, clearly demonstrates that the transfection and culture of human pluripotent cells (e.g., ES cells) was well known and predictable.

The evidence provides ample support that the Specification enables *in vitro* differentiation of pluripotent cells by inhibiting Smad4 and overexpressing a cell fate-inducing polypeptide. In view of the foregoing, it is respectfully submitted that the application is in condition for allowance and such action is respectfully requested.

Respectfully submitted,

Date 10/09/2007

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